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EXHIBIT B

The effects of a low frequency sonic waveform on peripheral vascular disease: A pilot study

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Word Count: 2952

Key Words: sonic waveform, alternative therapy, peripheral vascular disease, pain, intermittent claudication

Running Title: Effects of low frequency sound on peripheral vascular disease

SUMMARY

Objective: To evaluate the effects of a low frequency sonic waveform on peripheral vascular disease (PVD).

Design: Quasi-experimental pre-test, post-test pilot study.

Setting: Adults with PVD were recruited through local advertisements. The study was conducted at a local business complex.

Intervention: 25- minute exposure to a sonic waveform transmitted via the Cassone transducer.

Outcome Measures: Pre and post measurement of Doppler ultrasound arterial flow velocities, foot assessment, pain ratings, one-week post telephone survey.

Results: Significant differences were noted in the velocities of four out of 10 leg vessels, right ABI and pain ratings ($p < .05$). Thirteen participants reported PVD symptom improvement over the following week.

Conclusions: Exposure to low frequency sonic waveforms may be beneficial in short term relief of pain and decreased mobility associated with peripheral vascular disorders.

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INTRODUCTION

Peripheral vascular disease (PVD) is a disease of middle and older age, with symptoms appearing in men around age 45 and women by age 55-60.[1,2] Every two years, for the next 50 years, up to 1.3 million people can expect to develop disabling PVD in the U.S.[1] Symptoms vary with the extent of the disease. Intermittent claudication, pain felt in the calf while walking that is relieved with rest, is a hallmark symptom most frequently associated with PVD and is a strong diagnostic indicator of the disease.[3] The presence of intermittent claudication can severely limit one's ability to walk, ultimately leading to further impairment of peripheral circulation. Symptoms tend to worsen in 10-20% of people.[2] Staples of PVD treatment have included lifestyle change, vasoactive medications, and interventional radiological procedures or bypass surgery with grafting. The use of interventional procedures, such as arterial angioplasty or stint placement is limited to more severe cases. One year following angioplasty or stint placement, some 80-90% of patients maintain arterial patency.[3] Despite surgical and interventional advances, as many as 7% of all patients with PVD will go on to require amputation for critical limb ischemia within 5 years of symptom onset.[2]

The literature is rich with studies investigating the effects of various treatments on PVD. Studies involving lifestyle change have centered primarily on exercise and smoking cessation. Beard's[3] investigation of 21 exercise programs indicated that walking to the point of near maximum pain tolerance over a period of months significantly improved pain levels and walking distance. Ironically, many people have difficulty increasing their walking distance because of the pain associated with walking and the lack of improvement despite various treatment strategies. An analysis of three studies

regarding smoking cessation and peripheral vascular disease provided mixed results.[2] Results ranged from no difference in symptoms with smoking cessation to some improvement (though not statistically significant). The researchers also point out other modifications in lifestyle may have further confounded results. Davies[4] reported on two large-scale studies examining the role of smoking cessation on peripheral vascular disease symptoms. Of the 769 people participating in the studies, only 151 followed advice to quit smoking. In the following three years, there was no difference between the non-smoking and smoking groups in the incidence of limb threatening PVD complications.

Over the past several years, the use of medications to alter blood viscosity through decreased platelet aggregation in patients with PVD has been expanding. While cardiovascular benefits have been well documented, the research is not as conclusive in documenting the benefits on PVD disease symptoms.[4] A meta-analysis was performed on 13 pentoxifylline and six nafronyl studies to examine the effects of the drugs on PVD. Use of the medications demonstrated that, for some patients, walking time without pain increased by 20-60 minutes. However, ankle brachial index (ABI) readings, a standard, non-invasive indicator of PVD severity, were unchanged.[2]

Chappell and Stahl[5] conducted a meta-analysis of 19 studies in which ethylenediaminetetraacetic acid (EDTA) chelation therapy was used in the treatment of cardiovascular and PVD. The analysis revealed that 87% of participants experienced significant clinical improvement. One year later, Jonas[6] reported on the weakness of that meta-analysis in terms of study design (all but one of the studies lacked a control group and 94% of the participants represented only two of the 19 studies).

Unfortunately, none of these studies has provided clear, consistent evidence of PVD symptom improvement. This has continued to fuel the search for new methods and alternative therapies that may decrease the pain and disability so often associated with progressive PVD.

Effects of Sound

It wasn't until recently that healing effects from sonic waveforms in a specific low frequency range were anecdotally noted in people experiencing various diseases or injuries. As a result, research has been undertaken to study the effects of audible sound on disease, specifically PVD. Technically, sound is a form of vibration. Generically, sound and vibration are no different; both result from a disturbance of a medium (gas, solid, or liquid). Much research has been conducted on the effects of sound at various frequencies ranging from infrasound (inaudible less than 16 Hz) to ultrasound (inaudible greater than 20mHz). Although the range of human hearing is considered to be 16-20 kHz, few humans are capable of hearing beyond 10-12 kHz.[7] The common unit of measurement of sound pressure is the decibel (dB). It should be noted that dB is not a physical quantity, but rather a ratio of pressures.

Earlier investigations into the effects of sound on the body were performed throughout the 1960's and into the 1970's and revealed no detrimental effects or safety hazards from whole body exposure for short periods of time.[8-11] Much of the research conducted during this time was concerned with the physiological effects of sound on hearing. Some researchers found that prolonged exposure to noise levels above 90 dB resulted in hearing loss.[12] However, it soon became clear that hearing impairment was only one of many important effects that sound could have on the body. Many of the later

studies examined the physiological effects of sound on endocrine function in rats as well as humans, with some researchers finding increased activity of the corticoadrenal and adrenergic functions.[8,13,14] Other researchers investigated the effects of sound on inflammation. Billewicz-Stankiewicz and Krepinska-Urban[9] observed rats after a 2-hour exposure to 86 dB sound (sound and vibration) and reported an inhibition of the inflammatory response.

Although some older studies can be cited, few, if any, have been conducted in the past 20 years. The model work presented in many of these studies supports the idea that sound at low frequency levels is no threat to health except for a loss of hearing sensitivity in those studies that use excessively high decibel levels for prolonged periods of time. None of these studies examined the effects of vibration and low frequency audible sound on disease.

Electroacoustic transducers are widely used in many medical applications. Modem transducers often utilize the piezoelectric effect for converting electrical energy to acoustic or sonic energy. An alternating current applied to the material produces mechanical vibrations, in turn producing acoustic waves. Piezoelectric elements, therefore, are especially well suited to form the vibratory driving elements in electroacoustic transducers. Using new technology, the purpose of this study was to examine the effects of a low frequency sonic waveform on PVD.

METHODS

Sample

Adult volunteers were recruited from the community through local newspaper advertisements, which requested that interested people with PVD attend one of several scheduled 30-minute information sessions. Institutional review board approval and informed oral and written consents were obtained from eligible participants.

The major inclusion criterion was a history of medically diagnosed PVD. Exclusion criteria included: any previous sonic waveform treatments, implanted devices, pregnancy, use of birth control pills, major surgery or trauma in the past year, prolonged immobilization during the past six months, or history of deep vein thrombosis.

Design

The quasi-experimental research study utilized a one-group pretest-posttest design. Researchers emphasized the importance of continuing usual patterns of activity and exercise, diet, hydration, rest, and medications prior to the study.

On the day of the study, prior to the intervention, participants completed a brief background questionnaire along with a visual analog pain scale. Arm and ankle blood pressures were obtained to determine baseline ABI measurements. Lower extremity assessment included skin temperature, color, sensation, and range of motion. Utilizing a Doppler flow ultrasound, both legs were examined by a certified ultrasound technician. The common femoral, superficial femoral, popliteal, tibial, and dorsalis pedis arteries were measured for flow velocity. Following these pre-measurements, participants were escorted to a room for the treatment.

The treatment consisted of sitting in a chair for 25 minutes during which time the sonic wave generator was turned on. Participants were asked to wear headphones through which relaxation music could be controlled by individual volume dials. Post measurements were taken for the same variables following the treatment. A one-week follow-up telephone survey was conducted to assess any changes in PVD symptoms following the session.

Equipment

A portable Doppler ultrasound unit was used to measure blood flow velocity. The noninvasive ultrasound has become standard in assessing vessel function. An ultrasound technician placed conductive gel on the area of vessel to be examined and applied a hand-held transducer. The transducer contains two piezoelectric elements; one to transmit high frequency ultrasound waves (usually between 5-15 MHz) and one element to receive returning sounds that result from backscattering as sound hits red blood cells. The velocity is recorded on a screen in waveform patterns that correlate with flow centimeters per second. The pulsed flow ultrasound is not considered to be 100% exact because blood flow is pulsatile and is not uniform across the vessel. However, the calculated mean frequencies are highly reliable and are considered diagnostic in terms of evaluating the extent of peripheral artery disease (available online: www.mcbroom.btinternet.co.uk/medict/frames/Title.htm).

The treatment was performed using the Cassone Transducer. It consists of a metal cylinder with a slot extending its axial length and includes an arrangement of hollow piezoelectric cylinders, placed one atop another, to form a piezoelectric stack. The stack is tightly contained within a resilient metal sleeve. The sleeve and the

cylinders forming the stack, therefore, vibrate together as a unit when electric pulses are applied across the cylinders forming the stack. Ceramic material bonded to the tube's inner wall and having piezoelectric characteristics provides the driving mechanism in achieving wall vibrations.

The piezoelectric stack and the surrounding metal sleeve are equal in length. A gap or slot of narrow width is formed in both the stack and the sleeve, and extends axially along their lengths. The sleeve gap is coextensive, and aligned, with the gap in the piezoelectric stack. The combination of the piezoelectric stack and the sleeve, therefore, together form a highly stylized horseshoe, capable of vibrating like a tuning fork. The width of the gap opening (its circumferential length) affects the resonant frequency of the transducer, as does the piezoelectric wall thickness and the diameter of the stack. The piezoelectric cylinder is polarized radially, i.e., from the interior surface of the piezoelectric ceramic cylinder to its outside surface. The electrical pulses needed to cause vibrations of the cylinder are applied between the interior of the piezoelectric stack and the exterior of the steel sleeve.

Design frequencies at which the tube vibrates are directly related to the wall material, its thickness, the diameter, and to some extent, the width of the gap. A key facet of this technology is the efficiency in which electrical energy is converted to mechanical movement. The transducer is capable of operating at high efficiency, while resonating at a specified frequency. Additionally, it resonates with an omnidirectional beam pattern. The frequency used is this study is below 2000 Hz. We are unable to disclose the actual frequency range used in this study as it is considered proprietary at this time due to patent pending.

Instruments

A background questionnaire was developed by the researchers to gather information on age, gender, length of time diagnosed with PVD and symptoms associated with PVD. A brief follow-up survey was designed to assess PVD symptoms after one week. The instruments were pre-tested on 5 adult members of the community for readability.

A standard, 10-centimeter visual analog scale was utilized to assess PVD pain before and after the intervention. The visual analog scale ranged from 0 (no PVD pain) to 10 (worst PVD pain ever). Participants were asked to indicate a point along the line that best reflected pain felt at that moment. Visual analog scales have been widely used to assess pain and are considered valid and reliable in assessing perceived pain intensity.[15]

Data Analysis

Blood flow data were analyzed using the SPSS statistical package for Windows®, release 10.0. Means and standard deviations were calculated for the outcome variables. Independent t-tests were used to compare differences in outcome variables between pre and post-test means. Alpha levels were set at 0.05.

RESULTS

Fifteen people meeting the criteria participated in the study. The 10 males and 5 females had a mean age of 73.9 ± 10.6 years. The length of time participants had been medically diagnosed with PVD ranged from one year to longer than 20 years, with a mean of 8.5 years. All but one of the participants reported a variety of PVD symptoms including

pain, numbness, tingling, muscle fatigue, squeezing pressure, cramps, and swelling. Walking and standing were the positions most frequently reported to cause discomfort.

Incomplete data exist for several of the dependent variables secondary to the severity of disease and the difficulty in obtaining readings on many of the involved vessels. Information on participants with complete data is summarized in Tables 1 and 2.

Comparison of Data

Comparisons of pre and post data for each of the vessels examined are reported in Table 1. The means of all participants' demonstrated changes in blood flow for all of the vessels examined. However, significant differences were found between pre and post measures for only 4 of the 10 vessels investigated: Left common femoral artery (LCF) ($p = 0.022$), Right popliteal artery (RPOP) ($p = 0.016$), Left superior femoral artery (LSF) ($p = 0.000$), and Left posterior tibial artery (LPOSTIB) ($p = 0.045$).

Participants demonstrated increased mean scores for the left and right ankle brachial indexes (LABI and RABI, respectively), but exhibited significantly greater scores only for the RABI ($p = 0.05$). Significant decreases in pain were also noted between pre and post pain measures ($p = 0.045$). The LABI and RABI as well as pain data are reported in Table 2.

Little change was noted between the pre and post foot assessment. Three of the 15 participants experienced a change in foot color from pale to flesh color. Additionally, foot temperature improved from cool to warm on three participants. Lastly, three participants who initially felt no sensation to sharp touch were able to perceive the sharp sensation following the intervention.

Changes noted between pre-intervention symptoms and one-week post intervention symptoms are summarized in Table 3. Of the 15 participants, 13 reported improvement in one or more PVD symptoms during the week following the intervention. Prior to the intervention, there were 23 reports of leg pain, numbness, or tingling. One week after the intervention, that number had dropped to 15, an overall reduction of 35%.

Participants were asked to identify the positions causing the most leg discomfort. Walking and standing were the most identified positions of pain, followed by sitting and lying down. In the one-week telephone follow up, complaints of position discomfort with walking had decreased 25%, while standing and sitting position complaints had both dropped by 50%. For the one participant who experienced discomfort while lying down, no change was noted after one week. The distance that participants were able to walk before feeling PVD symptoms ranged from across the room to more than one block. Before the intervention, only five participants reported being able to walk more than one block before feeling PVD symptoms. One week later, nine participants reported that they could walk more than one block, an increase of 80%. The duration of symptom improvement ranged from 2-5 days, with one participant reporting improvement in walking distance and pain reduction for one full month.

DISCUSSION

The present study was designed to evaluate the effects of a low frequency sonic waveform on PVD. We demonstrated that exposure to the sonic waves at the designated frequency significantly increased the RABI as well as the blood flow velocity in four of the 10 vessels examined. Additionally, the intervention significantly decreased pain. We

are unable to corroborate the results obtained with other studies because of the novelty of this intervention. Studies involving infrasound (i.e. under 16 Hz) have yielded diverging results. Some of these researchers examined blood pressure changes during infrasonic exposure, reporting increased diastolic and decreased systolic pressures,[16] while others explored the effects of infrasound on the auditory and non-auditory systems.[10, 17-20] However, the results of these studies cannot be extrapolated to the present study because frequency and pressure ratios (decibels) are very different.

The characteristics of sonic frequency involved in the physiological reactions are mostly unknown. It is believed that peripheral vascular disorders involve changes in vessel structure and function. The changes in arterial velocity flow, ABI, and pain reports following the intervention suggest the possibility of transient changes in the vessel. It is also reasonable to question if the reports of symptom alleviation in the days following the intervention may involve some continued variation within the vessel. This pure speculation would require extensive studies utilizing invasive testing aimed at measuring specific indicators thought to be involved in vessel structure or function.

The study had several limitations. The small sample size and lack of control group make any generalizing of results to larger populations difficult. There are numerous factors that affect blood flow that the study did not attempt to control for, including underlying vessel size, stress level and endogenous hormone release, heart rate, cardiac output, hydration status, use of medications, diet, smoking status, use of herbs or vitamins, or the presence of concurrent diseases. External temperature was controlled as all participants were in the same building for the duration and the thermostat was set at

78° Fahrenheit. However, internal body temperatures may have differed from participant to participant.

The follow-up telephone survey relied on participant recall of subjective information. While useful, this type of information would be strengthened through the use of more objective data, such as follow up ultrasound, ABI, and nursing assessment of the feet.

Based on these preliminary findings, a larger scale study has begun which is double blinded and utilizes randomized control and treatment groups. The study should yield more objective information in determining the sound wave effects in alleviating peripheral vascular disease symptoms.

References:

1. Goodall S. Peripheral vascular disease. Nurs Stand 2000; 14(25): 48-52.

2. Girolami B, Bernardi E, Prins MH, Ten Cate JW, Hettiarachchi R, Prandoni P, et al. Treatment of intermittent claudication with physical training, smoking cessation, pentoxifylline, or nafronyl: a meta-analysis. Arch Intern Med 1999; 159(4): 337-345.

3. Beard JD. ABC of arterial and venous disease: Chronic lower limb ischaemia. Br Med J 2000; 320(7238): 854-857.

4. Davies A. The practical management of claudication. Br Med J 2000; 321(7266): 911-912.

5. Chappell LT, Stahl JP. The correlation between EDTA chelation therapy and improvement in cardiovascular function: a meta-analysis. J Adv Med 1993; 6: 139-160.

6. Jonas WB. Meta-analysis of EDTA chelation: Math that doesn't matter. J Adv Med 1994; 7: 109-111.

7. Kryter KD. The Effects of Noise on Man. New York: Academic Press; 1970.

8. Arguelles AE, Martinez MA, Pucciarelli E, Disisto MV. Endocrine and metabolic effects of noise in normal, hypertensive and psychotic subjects. In: Welch B L, Welch AS, eds. Physiological Effects of Noise. New York: Plenum Press, 1970: p. 43-55.

9. Billewicz-Stankiewicz J, Krepinska-Urban A. The effect of vibration and noise on development of inflammatory reaction in rats. Acta Physiol Pol 1974; 25(3): 235-240.

10. Borg E. Physiological and pathogenic effects of sound. Acta Otolaryngol Suppl 1981; 381: 1-68.

11. Graham FK, Slaby DA. Differential heart rate changes to equally intense white noise and tone. Psychophysiol 1973; 10(4): 347-362.

12. Rosen S. Noise, hearing and cardiovascular function. In: Welch B L, Welch AS, eds. Physiological Effects of Noise. New York: Plenum Press, 1970: p. 57-66.

13. Arguelles AE, D. I, Ottone JP, Chekherdemian M. Pituitary-adrenal stimulation by sound of different frequencies. J Clin Endocrin 1962; 22: 846.

14. Beardwood CJ, Mundell CA, Utian WH. Gonadotropin excretion in response to audiostimulation of human subjects. Am J Obstet Gynecol 1975; 121(5): 682-687.

15. Carlsson AM. Assessment of chronic pain. I. Aspects of the reliability and validity of the visual analogue scale. Pain 1983; 16(1): 87-101.

16. Danielsson A, Landstrom U. Blood pressure changes in man during infrasonic exposure. An experimental study. Acta Med Scand 1985; 217(5): 531-535.

17. Slarve RN, Johnson DL. Human whole-body exposure to infrasound. Aviat Space Environ Med 1975; 46(4 Sec 1): 428-431.

18. Hammelburg E. Biological effects of sound waves. Prog Biometeorol 1974; 1(1A): 409-412.

19. Borg E. Physiological aspects of the effects of sound on man and animals. Acta Otolaryngol Suppl 1979; 360: 80-85.

20. Borg E. Noise, hearing and hypertension. Scand Audiol 1981; 10(2): 125-126.

TABLE 1

Leg Blood Flow Velocity Values

Artery	Pre Mean	S.D.	Post Mean	S.D.	P-value
RCF (N=15)	85.20	39.43	102.67	42.04	0.065
LCF (N=15)	80.11	42.91	101.87	52.50	0.022
RDP (N=13)	42.83	18.19	47.29	19.14	0.451
LDP (N=15)	48.45	31.88	58.00	44.40	0.122
RPOP (N=14)	48.73	27.42	58.29	32.37	0.016
LPOP (N=15)	49.39	24.28	60.03	33.16	0.135
RSF (N=14)	82.53	42.91	88.86	45.82	0.433
LSF (N=14)	69.35	40.04	85.17	47.75	0.000
RPOSTIB (N=8)	66.56	46.95	72.79	50.72	0.054
LPOSTIBA (N=6)	56.33	40.61	78.47	26.99	0.045

R – right leg, L – left leg

C - common, F - femoral, D - dorsalis, P - pedis, POP - popliteal, S - superior,

POS - posterior, TIB - tibial

TABLE 2

Ankle Brachial Index and Pain Values

Index	Pre Mean	S.D.	Post Mean	S.D.	P-value
RABI (N=13)	.90	.36	1.00	.28	0.050
LABI (N=15)	.85	.35	.93	.35	0.246
Pain (N=15)	1.19	1.98	.53	1.25	0.045

R - right, L - left, ABI - ankle brachial index

TABLE 3

Self R port of PVD Symptomology

Indicator	Pre (# reporting)	One Week Post* (# reporting)
Symptoms felt in legs:		
Pain	10	7
Numbness	8	5
Tingling	5	3
Position(s) associated with discomfort:		
Walking	12	9
Sitting	2	1
Standing	8	4
Lying Down	1	1
Distance walked before feeling symptoms:		
Across room	2	1
1/2 block	4	1
one block	3	2
more than one block	5	9

*Duration of symptom improvement ranged from 2-5 days after intervention, one report lasting one full month

We would like to acknowledge the assistance provided by Alphonse Cassone and his facility in which this study was conducted. Mr. Cassone is the inventor of the Cassone Transducer, a patented technology. Mr. Cassone has applied for various medical patents utilizing the Cassone Transducer.